

WHAT IS CLAIMED IS:

1 1. A method of oxidizing a phosphite ester linkage in a nucleic acid
2 array to a phosphate linkage, comprising contacting said phosphite ester linkage with a
3 solution of from about 0.005 M to about 0.05 M iodine in a mixture of water and organic
4 solvent.

1 2. A method of preparing a nucleic acid array on a support, wherein
2 each nucleic acid occupies a separate known region of the support, said synthesizing
3 comprising:

4 (a) activating a region of the support;

5 (b) attaching a nucleotide to a first region, said nucleotide having a
6 masked reactive site linked to a protecting group;

7 (c) repeating steps (a) and (b) on other regions of said support whereby
8 each of said other regions has bound thereto another nucleotide comprising a masked
9 reactive site link to a protecting group, wherein said another nucleotide may be the same
10 or different from that used in step (b);

11 (d) removing the protecting group from one of the nucleotides bound to
12 one of the regions of the support to provide a region bearing a nucleotide having an
13 unmasked reactive site;

14 (e) binding an additional nucleotide to the nucleotide with an unmasked
15 reactive site;

16 (f) repeating steps (d) and (e) on regions of the support until a desired
17 plurality of nucleic acids is synthesized, each nucleic acid occupying separate known
18 regions of the support;

19 wherein said attaching and said binding are each made by covalently forming a
20 phosphite triester linkage between said nucleotides and said unmasked reactive site and
21 further comprising oxidizing said phosphite triester linkage to a phosphate triester linkage
22 with a solution of from about 0.005 M to about 0.05 M iodine in an aqueous solvent
23 mixture.

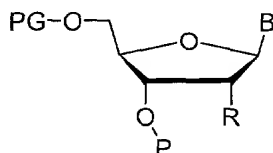
1 3. A method in accordance with claim 2, wherein said synthesizing
2 comprises the sequential steps of:

3 a) removing a photoremoveable protecting group from at least a first area
4 of a surface of a substrate, said surface comprising immobilized nucleotides on said

5 surface, said nucleotides capped with a photoremovable protective group, without
 6 removing a photoremoveable protecting group from at least a second area of said surface;
 7 b) simultaneously contacting said first area and said second area of said
 8 surface with a first nucleotide to couple said first nucleotide to said immobilized
 9 nucleotides in said first area, and not in said second area, said first nucleotide capped with
 10 said photoremovable protective group;
 11 c) removing a photoremoveable protecting group from at least a part of
 12 said first area of said surface and at least a part of said second area;
 13 d) simultaneously contacting said first area and said second area of said
 14 surface with a second nucleotide to couple said second nucleotide to said immobilized
 15 nucleotides in at least a part of said first area and at least a part of said second area;
 16 e) performing additional irradiating and nucleotide contacting and
 17 coupling steps so that a matrix array of at least 100 nucleic acids having different
 18 sequences is formed on said support;
 19 with the proviso that the coupling steps further comprise oxidizing an
 20 initially formed phosphite ester linkage to a phosphate ester linkage using from about
 21 0.005 M to about 0.05 M iodine in an aqueous solvent mixture.

1 4. A method in accordance with claim 3, wherein said aqueous
 2 solvent mixture comprises iodine in an amount of about 0.02 M.

1 5. A method in accordance with claim 3, wherein said nucleotides
 2 have the formula:



3 wherein

4 B is a member selected from the group consisting of natural or unnatural
 5 adenine, natural or unnatural guanine, natural or unnatural thymine,
 6 natural or unnatural cytosine, and natural or unnatural uracil;
 7 R is a member selected from the group consisting of hydrogen, hydroxy,
 8 protected hydroxy, halogen and alkoxy;
 9 P is a phosphoramidite group; and
 10 PG is a photoremoveable protected group.

1 6. A method in accordance with claim 5, wherein B is selected from
2 the group consisting of adenine, guanine, cytosine and thymine and R is hydrogen.

1 7. A method in accordance with claim 5, wherein said array
2 comprises at least 10 different nucleic acids.

1 8. A method in accordance with claim 5, wherein said array
2 comprises at least 100 different nucleic acids.

1 9. A method in accordance with claim 5, wherein said array
2 comprises at least 1000 different nucleic acids.

1 10. A method in accordance with claim 5, wherein said array
2 comprises at least 10,000 different nucleic acids.

1 11. A method in accordance with claim 5, wherein said array
2 comprises at least 100,000 different nucleic acids.

1 12. A method in accordance with claim 5, wherein each different
2 nucleic acid is in a region having an area of less than about 1 cm².

1 13. A method in accordance with claim 5, wherein each different
2 nucleic acid is in a region having an area of less than about 1 mm².

1 14. A method in accordance with claim 5, wherein said solution is
2 about 0.02 M iodine in a mixture of water, pyridine and THF.

1 15. A method in accordance with claim 5, wherein B is selected from
2 the group consisting of adenine, guanine, cytosine and thymine, R is hydrogen, and said
3 solution is about 0.02 M iodine in a mixture of water, pyridine and THF.

1 16. A method in accordance with claim 5, wherein B is selected from
2 the group consisting of adenine, guanine, cytosine and thymine, R is hydrogen, PG is
3 MeNPOC and said solution is about 0.02 M iodine in a mixture of water, pyridine and
4 THF.

1 17. A method in accordance with claim 5, wherein B is selected from
2 the group consisting of adenine, guanine, cytosine and thymine, R is hydrogen, PG is

- 3 MeNPOC, P is $-P(OCH_2CH_2CN)N(iPr)_2$ and said solution is about 0.02 M iodine in a
4 mixture of water, pyridine and THF.

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